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(54) Title: RETINOYLOXY(SUBSTITUTED)ALKYLENE BUTYRATES USEFUL FOR THE TREATMENT OF CANCER AND OTHER PROLIFERATIVE DISEASES

(57) Abstract

This invention relates to the novel retinoyloxy(substituted)alkylene butyrate compounds and pharmaceutical compositions containing same, to methods of treating, preventing or ameliorating cancer and other proliferative diseases in a subject in need of such treatment by comprising administering those compounds, pharmaceutically-acceptable salts or prodrugs thereof to a patient. The compounds of the invention are also useful in methods of inhibiting histone deacetylase, ameliorating wrinkles, treating or ameliorating dermatological disorders, inducing wound healing, treating cutaneous ulcers and treating gastrointestinal disorders.

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Retinoyloxy(substituted)alkylene butyrates Useful for the Treatment of Cancer and other Proliferative Diseases

The present invention is directed to retinoyloxy(substituted)alkylene butyrates and pharmaceutically acceptable salts thereof, to pharmaceutical compositions comprising said compounds, and to methods of treating cancer and other proliferative diseases in a subject in need of such treatment. The compounds of the invention are also useful in methods of inhibiting histone deacetylase, ameliorating wrinkles, treating or ameliorating dermatological disorders, inducing wound healing, treating cutaneous ulcers and treating gastrointestinal disorders.

BACKGROUND OF THE INVENTION

The uncontrolled proliferation of cancer cells is accompanied by the increase of immature cell populations. (Bloch, A., Cancer Treat. Rev. 68:199-205, 1984.) Although cancer cells are characterized by a marked capacity to proliferate and a limited capacity to differentiate under normal homeostatic conditions, experimental evidence has demonstrated that neoplastic cells can be induced to differentiate, indicating that malignant processes can be altered or, at least partially, reversed.

The retinoids are a family of compounds consisting of vitamin A, retinoic acid (RA) and related derivatives. They play a pivotal role in normal development of endodermally-, mesodermally- and ectodermally-derived tissues. (Umesono K. *et al.*, Nature 336:262-265, 1989).

The mode of action of RA has been extensively studied. In the cytoplasm, RA binds to the cytoplasmic RA-binding protein, whose role in mediating RA effects is unclear. In the nucleus, RA binds to the RA receptors (RAR-α, -β, -γ). The RAR/RA complex binds to a specific DNA sequence, as demonstrated by electrophoretic mobility

shift (Rochette-Egly *et al.*, J. Cell. Biol. 115:535-545, 1991), leading to transcription of RA target genes. RAR-α has been shown to be involved with growth and differentiation of myeloid cells *in vitro*. For instance, patients with acute promyelocytic leukemia have a characteristic (15:17) translocation with the breakpoint in the region of RAR-α coding sequence. Thus, mRNA for RAR are a useful tool to study potential anti-tumor drugs.

RA was reported to induce differentiation and arrest proliferation in a wide spectrum of cancer cells *in vitro* and *in vivo*, including patients with leukemia, myelodysplastic syndromes and solid tumors. For instance, Collins *et al.* Int. J. Cancer 25:213-218, 1980, have shown that the human promyelocytic leukemia cell line HL-60 can be induced to differentiate by RA and express cellular and molecular characteristics of granulocytes. (Umesono *et al.*) Emergence of differentiated features includes elevated protein kinase C and intracellular lysosomal activities. Strickland *et al.*, have shown that exposure of the teratocarcinoma cell line F9 to RA caused differentiation to visceral endoderm (Cell 15:393-403, 1978).

Several retinoids have achieved significant activity in the reversal of head and neck, skin, and cervical premalignancy and in prevention of second primary tumors associated with head and neck, skin and non-small lung cancer. Lippman *et al.* (J. Cell. Biochem. 22:1, 1995) have demonstrated chemoprevention activity of retinoids in aerodigestive tract carcinogenesis. This was tested in the two-stage mouse lung carcinogenesis model described by Nishimo, J. Cell. Biochem. 22:231, 1995.

Strickland *et al.*, demonstrated that in an *in vivo* murine model, orally administered RA increased the survival of mice bearing F9 tumors in a dose-dependent

manner (Dev. Biol. 78:76-85, 1980). The tumors of the RA-treated mice were much smaller in comparison to untreated animals and showed morphological and biochemical evidence of differentiation.

Because of the low therapeutic index of RA, its isomer, all-*trans* RA (ATRA), has been extensively studied. At 1 μm, ATRA has been shown to cause differentiation *in vitro* as demonstrated by measuring an increase of nitro-blue tetrazolium (NBT) reduction (Chomienne *et al.*, Blood 76:1710-1717, 1990).

Evidence also has accumulated for *in vivo* induction of differentiation by ATRA treatment. One morphological feature indicating differentiation of promyelocytic leukemia cell populations to mature cells is the appearance of Auer rods (homogenous crystallinic red-stained structure). Treatment of acute promyelocytic leukemia (APL) patients with ATRA resulted in complete remissions without bone-marrow hypoplasia. The presence of Auer rods in the maturing cells of these patients confirmed the differentiating activity of ATRA.

Due to results from clinical and laboratory studies, ATRA is now considered to be a first line therapeutic agent for promyelocytic leukemias (Wright D.G., Blood 67:334-337, 1987). However, the achievement of remission induced by ATRA tends to be brief and may be explained by rapid clearance in patients resistant to ATRA (Muindi *et al.* Cancer Res. 52:2138-2142, 1992). Moreover, Adamson *et al.* reports that patients orally administrated ATRA had highly variable absorption of the drug (J. Natl. Can. Inst., 85(12):993-996, 1993). Hence, maintenance of effective plasma concentrations and toxicity are problems associated with retinoid treatments (Adamson *et al.*, J. Natl. Cancer Inst. 85:993-996, 1993).

Butyric acid (BA) is a non-toxic natural product. It is supplied to mammals from two main sources: 1) the

diet, mainly from dairy fat, 2) as a major product of bacterial fermentation of unabsorbed carbohydrates in the colon, where it reaches mM concentrations (Cummings J.H., Gut 22:763-779, 1982; Leder A. *et al.*, Cell 5:319-322, 1975).

BA has been known for nearly the last three decades to be a potent differentiating and antiproliferative agent in a wide spectra of neoplastic cells *in vitro* (Prasad N.K., Life Sci. 27:1351-1358, 1980). In cancer cells, BA is reported to induce cellular and biochemical changes, e.g., in cell morphology, enzyme activity, receptor expression and cell-surface antigens (Nordenberg J. *et al.*, Exp. Cell Res. 162:77-85, 1986; Nordenberg J. *et al.*, Br. J. Cancer 56:493-497, 1987; and Fishman P.H. *et al.*, J. Biol. Chem. 254:4342-4344, 1979).

Although BA or its sodium salt (sodium butyrate, SB) has been the subject of numerous studies, its mode of action is unclear. The most specific effect of butyric acid is inhibition of nuclear deacetylase(s), resulting in hyper acetylation of histones H3 and H4 (Riggs M.G., *et al.*, Nature 263:462-464, 1977). Increased histone acetylation, following treatment with BA has been correlated with changes in transcriptional activity and the differentiated state of cells (Thorne A.W. *et al.*, Eur. J. Biochem. 193:701-713, 1990). BA also exerts other nuclear actions, including modifications in the extent of phosphorylation (Boffa L.C. *et al.*, J. Biol. Chem. 256:9612-9621, 1981) and methylation (Haan J.B. *et al.*, Cancer Res. 46:713-716, 1986). Other cellular organelles, e.g., cytoskeleton and membrane composition and function, have been shown to be affected by BA (Bourgeade M.F. *et al.*, J. Interferon Res. 1:323-332, 1981). Modulations in the expression of oncogenes and suppressor genes by BA were demonstrated in several cell types. Toscani *et al.*, reported alterations in c-myc,

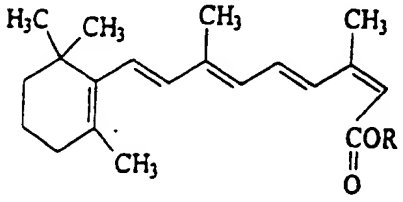
p53 thymidine kinase, c-fos and AP2 in 3T3 fibroblasts (Oncogene Res. 3:223-238, 1988). A decrease in the expression of c-myc and H-ras oncogenes in B16 melanoma and in c-myc in HL-60 promyelocytic leukemia were also reported (Prasad K.N. *et al.*, Biochem. Cell Biol. 68:1250-1255, 1992; and Rabizadeh E. *et al.*, FEBS Lett. 328:225-229, 1993). However, BA is normally metabolized rapidly and has a very short half-life *in vivo*. Thus the achievement and maintenance of effective plasma concentrations are also problems associated with BA.

BA is known to induce cell death via apoptosis. Apoptosis is the physiological mechanism for the elimination of cells in a controlled and timely manner. Organisms maintain a delicate balance between cell proliferation and cell death, which when disrupted can tip the balance between cancer, in the case of over accumulation of cells, and degenerative diseases, in the case of premature cell losses. Hence, inhibition of apoptosis can contribute to tumor growth and promote progression of neoplastic conditions.

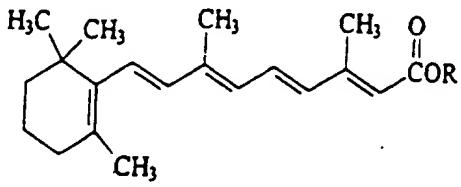
Synergistic anti-proliferative and differentiating effects of combinations of RA with other differentiating agents or cytokines have been suggested. For instance, Breitman *et al.*, demonstrated that BA alone induced differentiation of HL-60 cells, a human promyelocytic cell line, with an ED50 of 444 μM, and RA alone induced HL-60 cells with an ED50 at 0.13 μM. However, the combination of about 28 nM RA with BA reduced the ED50 value for BA from about 400 to about 75 μM, a dose reduction index value of about 6-fold (Cancer Res. 50:6268-6273, 1990). Based on his study, Breitman suggested that RA might be useful in combination with other agents in the treatment of some leukemias. However, treatment with either BA or RA alone or in combination will continue to have the problems of

toxicity, as well as achieving and maintaining effective plasma concentrations.

Others have studied conjugated RA compounds. For instance, Parish, U.S. Patent No. 4,677,120 (issued June 30, 1987) and PCT Application No. WO 90/06751 disclose the use of compounds of formulas A or B:



(A) CIS



(B) TRANS

wherein R is $CR_2'''OC(=O)CR_3'$, R' is H or C_1-C_6 alkyl, R''' is R' or the hydrocarbon backbone of fatty acids, for affecting the reduction and reversal of photo aging and skin cancer. This application does not disclose or enable methods of the present invention.

Gross, U.S. Patent No. 4,900,478 (issued February 13, 1990), discloses, *inter alia*, all-*trans*-9-4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nona-tetraenoic acid and in EPO Application 0449099 discloses the use of compounds of the formula $R^1OCH(R^2)OC(O)R^3$, wherein R^1 is 13-*cis*-retinoyl, R^2 is alkyl and R^3 is alkyl or alkoxy, for the treatment of skin disorders. This application does not disclose or enable methods using the retinoyloxy(aryl-substituted)alkylene butyrates of the present invention.

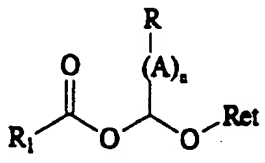
Thus, there remains the need to identify compounds as effective as the combination of BA and RA as differentiating or anti-proliferating agents for the

treatment of cancers. Such compounds need to have high potency without the problems associated with BA and RA.

This invention addresses this need and is thus directed to the compounds of Formula (I) which are retinoyloxy(substituted)alkylene butyrates and which are more potent than BA or RA alone or combined, to compositions comprising same and to methods of using same for the treatment of cancers and other proliferative diseases, for inhibiting histone deacetylase, for gastrointestinal disorders, for ameliorating wrinkles, for wound healing and for treatinbg dermatological disorders (in the case of the aryl-substituted compounds). None of the references discussed above teaches or suggests the compounds of Formula (I), pharmaceutical compositions containing same or the methods of using these compounds or compositions as anti-cancer and anti-proliferative agents.

SUMMARY OF THE INVENTION

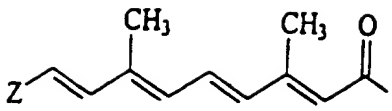
Accordingly, one embodiment of the present invention is directed to the novel compounds having the Formula (I):



Formula (I)

wherein:

Ret is selected from the group consisting of a retinoyl group, a therapeutically-active retinoid carbonyl group, a therapeutically-active carbonyl group represented by the formula



and retinoids which are C20 or C22 desmethyl vinylogs of said groups, wherein Z is a substituted or unsubstituted phenyl group, a substituted or unsubstituted naphthyl group or a cyclohexenyl group, and said phenyl or naphthyl group can be substituted with from 0 to 5 substitutents selected from the group consisting of halo, hydroxy, alkyl, alkyoxy, amino, cyano or carbalkoxy, and wherein double bonds in the polyene chain of any of said groups can have a *cis* or *trans* configuration;

n is 0 or 1; and

when n is 0, then

R is H or C_1 to C_5 alkyl,

R_1 is ethyl, *n*-propyl or isopropyl,

with the proviso that when Ret is 13-*cis*-retinoyl and R_1 is *n*-propyl, then R cannot be H or C_1 to C_5 alkyl; or

when n is 1, then

R is aryl or heteroaryl optionally substituted with halo, hydroxy, alkyl, alkoxy, amino, cyano, carbalkoxy, nitro, or trifluoromethyl,

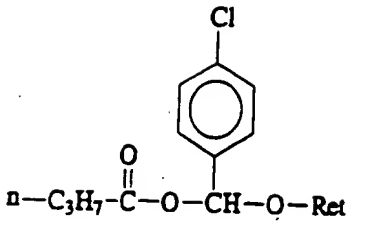
R_1 is C_1 to C_7 alkyl, optionally substituted by a phenyl or substituted phenyl group,

A is $(CH_2)_m$ or $(CH{=}CH)_m$,

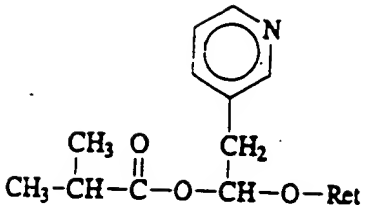
m is 0 to 4; and

pharmaceutically acceptable salts thereof.

Preferred are compounds wherein Ret is *trans*. Specifically preferred is the compound *trans*-retinoyloxymethylbutyrate (ROBA) as well as compounds of the general Formula II and III shown below:



II



III

where Ret is as herein defined. The compounds of Formula II are retinoyloxy(*p*-chlorophenyl)methyl butyrates and the compounds of Formula III are the pharmaceutically-acceptable anion salts of 1-retinoyloxy-2-(3-pyridyl)ethyl isobutyrate and its N-alkyl pyridinium salts. The compounds of the present invention have greater efficacy as proliferation inhibitors and differentiating agents than either BA or RA alone or a combination of BA plus RA.

Another embodiment of the present invention is drawn to pharmaceutical compositions comprising a therapeutically effective amount of a compound of Formula (I) and a pharmaceutically effective carrier or diluent.

A further embodiment of the present invention is directed to pharmaceutical compositions comprising a therapeutically effective amount of a combination a compound of Formula (I) with other anti-cancer or anti-neoplastic agents together with a pharmaceutically effective carrier or diluent.

Another embodiment of the present invention is directed to methods of treating, preventing or ameliorating cancer and other proliferative disorders which comprise administering a therapeutically effective amount of a compound of Formula (I) to a subject suffering from such disorders and to methods of enhancing the actions of other known pharmaceutical agents,

including antiproliferative, differentiating or oncostatic agents.

The pharmaceutical agents of the invention for the above method include, but are not limited to, cytokines, interleukins, anti-cancer agents, chemotherapeutic agents, antibodies, conjugated antibodies, immune stimulants, antibiotics, hormone antagonists, and growth stimulants. The compounds of the invention can be administered prior to, after or concurrently with any of the agents.

Yet another aspect of the invention is directed to a method of inhibiting histone deacetylase in cells by administering a compound of Formula I to said cells.

A still further embodiment of the invention is directed to a method of ameliorating wrinkles, inducing wound healing, treating cutaneous ulcers or treating a gastrointestinal disorder by administrating a therapeutically-effective amount of a compound of Formula(I) to a subject in need of such treatment. The cutaneous ulcers which can be treated in accordance with the methods of the invention include leg and decubitus ulcers, stasis ulcers, diabetic ulcers and atherosclerotic ulcers. With respect to wound healing, the compounds are useful in treating abrasions, incisions, burns, and other wounds. Gastrointestinal orders treatable by the methods of the invention include colitis, inflammatory bowel disease, Crohn's disease and ulcerative colitis.

Another embodiment of the invention is directed to a method of treating or ameliorating dermatological disorders by administrating a compound of Formula (I) wherein n is 1 to a subject in need of such treatment. In accordance with the invention, dermatological disorders include psoriasis, acne and the like.

Preferably the compounds are administered in topical preparations.

The methods of the present invention are particularly useful for treating, preventing or ameliorating the effects of cancer and other proliferative disorders by acting as anti-proliferative or differentiating agents in subjects afflicted with such anomalies. Such disorders include but are not limited to leukemias, such as acute promyelocytic leukemia, acute myeloid leukemia, and acute myelomonocytic leukemia; other myelodysplastic syndromes, multiple myeloma such as but not limited to breast carcinomas, cervical cancers, melanomas, colon cancers, Kaposi's sarcoma, ovarian cancers, pancreatic cancers, hepatocarcinomas, prostate cancers, squamous carcinomas, renal cell carcinoma, other dermatologic malignancies, teratocarcinomas, T-cell lymphomas, lung tumors, gliomas, neuroblastomas, peripheral neuroectodermal tumors, rhabdomyosarcomas, and prostate tumors and other solid tumors. It is also possible that compounds of Formula (I) have anti-proliferative effects on non-cancerous cells as well, and may be of use to treat benign tumors and other proliferative disorders such as psoriasis. Preferred is the method for treating or ameliorating leukemia, squamous cell carcinoma and neuroblastoma.

While it is possible to utilize the compounds *in vivo* as raw chemicals, it is preferable to present them as pharmaceutical compositions.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the effect of all-*trans* retinoids on cell differentiation in human HL-60 cells.

DETAILED DESCRIPTION OF THE INVENTION

The compounds herein described may have asymmetric centers. All chiral, diastereomeric, and racemic forms are included in the present invention. Many geometric isomers of olefins and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present invention.

By "stable compound" or "stable structure" is meant herein a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent.

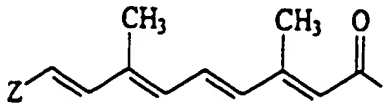
As used herein, "alkyl" includes both branched- and straight-chain, saturated or unsaturated aliphatic hydrocarbon groups having the specified number of carbon atoms. The alkyl groups of the invention have up to 7 carbon atoms, and preferably 1 to 5 carbon atoms or 1 to 7 carbon atoms.

As used herein, "aryl" is intended to mean any stable 5- to 7-membered monocyclic or bicyclic or 7- to 14-membered bicyclic or tricyclic carbon ring, containing at least one aromatic ring, for example, phenyl, naphthyl, indanyl and the like.

As used herein, the term "heteroaryl" is intended to mean a stable 5- to 7-membered monocyclic or bicyclic or 7- to 10-membered bicyclic heterocyclic ring which is aromatic, and which consists of carbon atoms and from 1 to 3 heteroatoms selected from the group consisting of N (nitrogen), O (oxygen) and S (sulphur) and wherein the nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen may optionally be quaternized, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached to its pendant group at any heteroatom or carbon atom which

results in a stable structure. The heterocyclic rings described herein may be substituted on carbon or on a nitrogen atom if he resulting compound is stable. Examples of such heterocycles include, but are not limited to, pyridyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, benzothiophenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl or benzimidazolyl.

As used herein a therapeutically active retinoid is a compound that exhibits a biological action similar to retinoic acid (i.e., similar to vitamin A acid). Hence retinoids include those compounds, synthetic or natural, which have one or more of the therapeutic activities known for retinoic acid. Such activities include but are not limited to binding to and activating retinoic acid receptors, treating and preventing cancer and other proliferative disorders, acting as differentiating agents or anti-proliferatives agents and anti-tumor activity. Thus, as embodied herein Ret of Formula (I) is a retinoid carbonyl group of a therapeutically-active retinoid. Moreover, Ret includes compounds of the formula



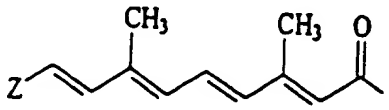
where Z is as defined above. Examples of retinoids contemplated by the invention can be found in U.S. Patent Nos. 4,476,056; 4,105,681; 4,215,215; 4,054,589 and 3,882,244. Retinoids include both *cis* and *trans* forms having therapeutic activity. Preferred retinoids include those having a 9-*cis* double bond, a 13-*cis* double bond or a 13-*trans* double bond.

The term "substituted", as used herein, means that one or more hydrogens on the designated atom are replaced with a selection from the indicated groups, provided that

the designated atom's normal valency is not exceeded, and that the substitution results in a stable compound.

The substituted aryl and heteroaryl groups of the invention have one or more hydrogen atoms replaced with a halo, hydroxy, alkyl, alkoxy, amino, cyano, carboxy carbalkoxy, nitro or trifluoromethyl group. A halo group is a halogen, and includes fluoro, chloro, bromo and iodo groups. The term alkoxy refers to an alkyl group having at least one oxygen substitutent. The term carbalkoxy refers to groups of the formula -R-C(O)O- where R is an alkyl group.

As used herein "vinylogs" are desmethyl retinoyl groups having 1 or 2 additional vinyl groups relative to retinoic acid. For example such compounds include 2,6,6,-trimethyl-1-(10'-carboxy-deca-1',3',5',7',9'-pentaenyl)cyclohex-1-ene and 2,6,6-trimethyl-1-(12'-carboxy-dodeca-1',3',5',7',9',11'-hexaenyl)cyclohex-1-ene. These groups are also referred to as C20 and C22 vinylogs of desmethyl retinoic acid and are described in U.S. Patent No. 3,882,244. The vinylogs of this invention can be prepared from a retinoyl group, any therapeutically active retinoid carboxyl group, or any group of the formula



wherein Z is as defined herein.

As used herein, "therapeutically-effective amount" refers to that amount necessary to administer to a host to treat, prevent or ameliorate cancer, or other proliferative disorder, wherein that amount can further be an amount effective to inhibit histone deacetylase in the cells of a patient; to achieve an anti-tumor effect; to induce differentiation and/or inhibition of

proliferation of malignant cancer cells, benign tumor cells or other proliferative cells; to aid in the chemoprevention of cancer; to achieve an anti-wrinkling effect; to treat or ameliorate psoriasis; to promote wound healing or to treat a gastrointestinal disorder. Therapeutically-effective amounts can be readily determined by one of ordinary skill in the art.

As used herein and in the claims, "pharmaceutically acceptable salts and prodrugs" refer to derivatives of the disclosed compounds that are modified by making acid or base salts, or by modifying functional groups present in the compounds in such a way that the modifications are cleaved, either in routine manipulation or *in vivo* in relation to the parent compounds. Examples include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; acetyl, formyl and benzoyl derivatives of amines; and the like.

Pharmaceutically-acceptable salts of the compounds of the invention are prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418, the disclosure of which is hereby incorporated by reference in its entirety.

The "pharmaceutical agents" for use in the methods of the invention related to the coadministration of compounds of Formula I, include but are not limited to anticancer agents as well as differentiating agents. For example, the pharmaceutical agent is a cytokine, an interleukin, an anti-cancer agent, a chemotherapeutic

agent, an antibody, a conjugated antibody, an immune stimulant, an antibiotic, a hormone antagonist or a growth stimulant. The pharmaceutical agent is a cytotoxic agent. Cytotoxic agents include antiviral nucleoside antibiotics such as ganciclovir, acyclovir, and famciclovir. Cytotoxic agents also include radiation therapy.

As used herein, the "chemotherapeutic agents" include but are not limited to alkylating agents, purine and pyrimidine analogs, vinca and vinca-like alkaloids, etoposide and etoposide-like drugs, corticosteroids, nitrosoureas, antimetabolites, platinum-based cytotoxic drugs, hormonal antagonists, anti-androgens and antiestrogens.

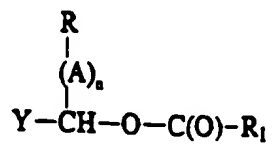
The "cytokines" for use herein include but are not limited to interferon, preferably α, β or γ interferon, as well as IL-2, IL-3, G-CSF, GM-CSF and EPO.

As used herein, an "immune stimulant" is a substance such as *C. parvum* or sarcolectin which stimulates a humoral or cellular component of the immune system.

The chemotherapeutic agents of the invention include but are not limited to tamoxifen, doxorubicin, L-asparaginase, dacarbazine, amsacrine, procarbazine, hexamethylmelamine, mitoxantrone and gemcitabine.

The compounds provided by the present invention are prepared generally by any method known in the art. Preparation of the compounds of the invention is illustrated by the following non-limiting example.

For example, the compounds of the invention can be made by reacting the acid form of the retinoid (e.g., Ret-OH) with a reagent of the formula



in the presence of a base, where Y is a leaving group such as halogen, methanesulfonate or *p*-toulenesulfonate and Ret, A, R and R_1 are as defined herein. The base is a trialkylamine, pyridine, an alkali metal carbonate or other suitable base. The reaction is carried out in the presence or absence of an inert solvent. When a solvent is used, it is, for example, acetone, benzene, toluene, tetrahydrofuran, ethyl acetate, acetonitrile, dimethylformamide, dimethyl sulfoxide, chloroform, dioxan or 1,2-dichloroethane. Additional methods and details for synthesis of the compounds of this invention are provided in Nudelman *et al.*, J. Med. Chem. 35:687-694, 1992.

The procedures outlined above can be improved by one skilled in the art by, for instance, changing the temperature, duration, stoichiometry or other parameters of the reactions. Any such changes are intended to fall within the scope of this invention.

The compounds of the present invention are generally useful in the treatment of indications including cancer and other proliferative disorders, as differentiating agents or antiproliferative agents and in the chemoprevention of cancer.

These activities are measured using generally-accepted techniques known to those skilled in the art. For instance, the activity of compounds useful as differentiating agents can be measured using standard methodology of the nitro-blue tetrazolium reduction assay (e.g., Rabizadeh *et al.*, FEBS Lett. 328:225-229, 1993; Chomienne *et al.*, Leuk. Res. 10:631, 1986; and Breitman *et al.* in Methods for Serum-free Culture of Neuronal and Lymphoid Cells, Alan R. Liss, NY, p. 215-236, 1984 which are hereby incorporated by reference in their entirety) and as described below. This *in vitro* assay has been deemed to be predictive and in fact correlative with *in*

vivo efficacy (Castaigne *et al.*, Blood 76:1704-1709, 1990).

Another assay which is predictive of differentiating activity is the morphological examination for the presence of Auer rods and/or specific differentiation cell surface antigens in cells collected from treatment groups, as described in Chomienne *et al.*, (Blood 76:1710-1717, 1990 which is hereby incorporated by reference in its entirety) and as described below.

The compounds of the present invention also have anti-proliferative and anti-tumor activity. The anti-proliferation activity of compounds of the present invention are determined by methods generally known to those skilled in the art. Two generally-accepted assays for measuring viability and anti-proliferative activity are the trypan blue exclusion test and incorporation of tritiated thymidine, also as described by Chomienne, *et al.*, above, which is incorporated herein by reference. Another assay which is predictive and is shown to correlate with antitumor activity and *in vivo* efficacy is the human tumor colony forming assay described in Shoemaker *et al.*, Can. Res. 45:2145-2153, 1985, which is incorporated herein by reference in its entirety. These assays are described in further detail below.

Cell Cultures

Human promyelocytic leukemia Cells (HL-60), Human Pancreatic Carcinoma Cells (PaCa-2) and Human Breast Adenocarcinoma, pleural effusion, Cells (MCF-7) can be cultured as follows. Cells are grown in RPMI media with 10% FCS, supplemented with 2 mM glutamine and incubated at 37°C in a humidified 5% $C0_2$ incubator. Viability is determined by trypan blue exclusion. Cells are exposed to butyric acid or retinoic acid or a compound of the

invention and cultures are harvested at various time points following treatment.

Nitro-Blue Tetrazolium (NBT) Assay:

Cell differentiation is evaluated by NBT reduction activity as follows. Cell cultures containing 0.1% NBT are stimulated with 400 nM of 12-O-tetradecanoyl-phorbol-13-acetate (P.A.). The cells are incubated for 30 min at 37°C and examined microscopically by scoring at least 200 cells. The capacity for cells to reduce NBT is assessed as the percentage of cells containing intracellular reduced black formazan deposits and corrected for viability.

Additional Cell Differentiation Experiment

Human promyelocytic cell line HL-60 are grown for 4 days in the presence of 0.25 μM of RA, or a retinoyloxy(substituted)alkylene butyrate of the invention. The compounds are synthesized as described above. Cell differentiation is measured by the NBT assay described above.

Cell Surface Antigen Immunophenotyping

Cell surface antigen immunotyping are conducted using dual-color fluorescence of cells gated according to size. The expression of a panel of antigens from early myeloid (CD33) to late myeloid is determined as described in Warrell, Jr. *et al.*, New Engl. J. Med. 324:1385-1392, 1992, which is incorporated by reference herein in its entirety.

Northern Blot Analysis for RAR-α Expression

Total cellular RNA analysis is conducted by guanidinium thiocyanate phenol/chloroform extraction as described by Rabizadeh *et al.*, FEBS Lett. 328(3):225-229,

1993, and probed with human complementary DNA (cDNA) for RAR-α as described by Miller *et al.*, J. Natl. Cancer Inst. 82:1932-1933, 1990, which are incorporated by reference herein in their entireties.

Southern Blot Analysis for Genomic Rearrangements of RAR-α

Genomic DNA is prepared and completely digested for three hours with *Eco*R1 or *Hind*III (2-3 U per microgram DNA). DNA is then size fractionated on 0.8% agarose gel, denatured, renatured, neutralized and blotted onto a nitrocellulose filter. The filter is then hybridized to a 640-base pair *Eco*R1-*Sst*1 cut RAR-α cDNA and washed stringently at 55°C. Autoradiograms are obtained after exposure at -70°C to Kodak-XAR film with use of an intensifying screen.

Apoptosis Evaluation

Apoptosis can be evaluated by DNA fragmentation, visible changes in nuclear structure and immunocytochemical analysis of Bcl-2 expression.

DNA fragmentation is monitored by the appearance of a DNA ladder on an agarose gel. Cellular DNA is isolated by the method of Martin *et al.*, J. Immunol., 145:1859-1867, 1990. Briefly, cells are washed twice with PBS and centrifuged at 1200 rpm at room temperature for 5 min. The pellets are resuspended at $2x10^7$ cells/mL in lysing buffer (10 mM EDTA, 50 mM Tris, pH 8) containing 0.5% (w/v) N-laurylsarcosine and 0.5 mg/mL proteinase K and incubated for 1 h at 50°C, Heat-treated Rnase is added to a concentration of 0.25 mg/mL and incubation at 50°C continued for 1 h. The crude DNA preparations are extracted with buffered phenol followed by two chloroform:isoamyl alcohol (24:1) extractions. DNA preparation are brought to 2.5 volumes in 10 mM Tris, pH

8, 1 mM EDTA (TE buffer) and precipitated for 24 hours in 2 volumes of ethanol at -70°C. The DNA precipitates are recovered by centrifugation, air dried, resuspended in TE buffer, and stored at 4°C. DNA concentration is calculated by determining the OD at 260 nm. Electrophoresis of DNA is carried out in 1% agarose gels containing 1 μg ethidium bromide as described (Martin *et al.*). Gels contain size markers (Φx174 DNA *Hae*III digest, 11 fragments ranging from 72-1353 bp). After electrophoresis, stained gels are viewed by transillumination with UV light (302 nm) and photographed through a DS34 Polaroid direct screen instant camera using Polaroid 667 (3000 ASA) film.

Changes in nuclear structure are assessed by acridine orange staining method of Hare *et al.*, J. Hist. Cyt., 34:215-220, 1986. Cytospins are prepared from HL-60 cells treated with BA, RA, or with one of the compounds of the invention. Untreated cells are used as a control. Cells are fixed with 100% ethanol for 10 min. Acridine orange (1.2 mg/mL in 0.13 M Na_2HPO_4, 0.35 M citric acid and 1μM Na_2EDTA, pH 6.5) is applied to the fixed cells for 30 min. At least 3 fields containing about 250 cells are examined and counted under an Olympus BH-2 fluorescence microscope. The fields are photographed with an Olympus camera using Agfa film (ASA 1000).

Immunological detection of Bcl-2 is performed on untreated HL-60 cells or HL-60 cells treated with BA, RA or one of the compounds of RN-1 to RN-4. Cytospins are prepared and the cells are fixed with ethanol. Fixed cells are reacted overnight at 4°C with the primary monoclonal antibody anti-Bcl-2 (Dako) at a dilution of 1:50. Staining is completed using Strep A-B Universal Kit (DPC, Sigma) according to manufacturer's instructions. Microscopy and photography are performed

as in the preceding paragraph except that the film is ASA 200. Identically-treated cells which received no primary antibody serve as non-specific binding controls.

Mouse Cancer Model

Compounds of the present invention can be examined for their ability to increase the life span of animals bearing B16 melanomas, Lewis lung carcinomas and myelomonocytic leukemias as described in Nudelman *et al.*, J. Med. Chem. 35:687-694, 1992, or Rephaeli *et al.*, Int. J. Cancer 49:66-72, 1991, which are incorporated by reference herein in their entireties.

The efficacy of compounds of the present invention in the leukemia model is tested as follows: Balb/c mice are injected with WEHI cells and drug or control solution is administered the following day. The life span of the treated animals is compared to that of untreated animals.

The efficacy of compounds of the present invention on primary tumors is tested in subcutaneously implanted lung carcinoma or B16 melanoma by measuring the mass of the tumor at the site of implantation every two weeks in control and drug-treated animals.

Chemoprevention

The chemoprevention activity of the compounds of the invention is determined in the two-stage mouse carcinogenesis model of Nishimo *et al.* (supra).

Xenografts

Colon adenocarcinoma (human HCT-15 cells), mammary adenocarcinoma (human MX-1 cells) and melanoma (murine B16) xenografts are made by implanting the respective cells subcutaneously into athymic mice. Treatment with control solution or a compound of Formula (I) begins when

tumors are approximately 100 mg. Anti-tumor activity is assessed by the delay in tumor growth.

Compounds of Formula (I), their salts or metabolites, can be measured in a biological sample by any method known to those skilled in the art of pharmacology, clinical chemistry or the like. Such methods for measuring compounds of Formula (I) are standard methods and include, but are not limited to high performance liquid chromatography (HPLC), gas chromatography (GC), gas chromatography mass spectroscopy (GC-MS), radioimmunoassay (RIA), and others.

Dosage and Formulation

The compounds of the present invention are administered to treat cancer or other proliferating disorders by any means that produces contact of the active agent with the agent's site of action in the body of a subject. They are administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic agents or in a combination of therapeutic agents. They can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice. The pharmaceutical compositions of the invention can be adapted for oral, parenteral, transdermal or transmucosal administration, and may be in unit dosage form, as is well known to those skilled in the pharmaceutical art. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, or intrasternal injection or infusion techniques.

The appropriate dosage administered in any given case, of course, varies depending upon known factors, such as the pharmacodynamic characteristics of the particular agent and its mode and route of

administration; the age, general health, metabolism, weight of the recipient and other factors which influence response to the compound; the nature and extent of the symptoms; the kind of concurrent treatment; the frequency of treatment; and the effect desired. A daily dosage of active ingredient is expected to be about 0.001 to 500 milligrams per kilogram (mg/kg) of body weight, with the preferred dose being 0.01-50 mg/kg.

Dosage forms (compositions suitable for administration) contain from about 1 mg to about 1 g of active ingredient per unit. In these pharmaceutical compositions the active ingredient is ordinarily present in an amount of about 0.5-95% by weight based on the total weight of the composition.

The active ingredient can be administered orally in solid or semi-solid dosage forms, such as for example hard or soft-gelatin capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, disperse powders or granules, emulsions, and aqueous or oily suspensions. It can also be administered parenterally, in sterile liquid dosage forms. Other dosage forms are possible, such as but not limited to, administered transdermally, via a patch mechanism or ointment.

Compositions intended for oral use are prepared according to any methods known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including sweetening agents, flavoring agents, coloring agents, and preserving agents in order to provide a pharmaceutically elegant and palatable preparation.

Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. Such excipients include, for example, inert diluents, such as calcium phosphate, calcium carbonate, sodium carbonate,

sodium phosphate, or lactose; granulating disintegrating agents, for example, maize starch or alginic acid; binding agents, such as starch, gelatin, or acacia; and lubricating agents, for example, magnesium stearate, stearic acids or talc. Compressed tablets are uncoated or sugar coated or film coated by known techniques to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration and adsorption in the gastrointestinal tract.

Hard gelatin capsules or liquid filled soft gelatin capsules contain the active ingredient and inert powdered or liquid carriers, such as, but not limited to calcium carbonate, calcium phosphate, kaolin, lactose, lecithin starch, cellulose derivatives, magnesium stearate, stearic acid, arachis oil, liquid paraffin, olive oil, pharmaceutically-accepted synthetic oils and other diluents suitable for the manufacture of capsules. Both tablets and capsules can be manufactured as sustained release-products to provide for continuous release of medication over a period of hours.

Aqueous suspensions contain the active compound in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, e.g., sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth, and gum acacia; dispersing or wetting agents, such as a naturally occurring phosphatide, e.g., lecithin, or condensation products of an alkylene oxide with fatty acids, for example of polyoxyethylene stearate, or a condensation products of ethylene oxide with long chain aliphatic alcohols, e.g., heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol, e.g.,

polyoxyethylene sorbitol monooleate, or a condensation product of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, e.g., polyoxyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, *n*-propyl, or *p*-hydroxy benzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin, or sodium or calcium cyclamate.

Disperse powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example, sweetening, flavoring, and coloring agents, can also be present.

Syrups and elixirs are formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents.

The pharmaceutical compositions can be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous suspension. This suspension is formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation can also be a sterile injectable solution or suspension in a nontoxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol.

In general, water, a suitable oil, saline, aqueous dextrose (glucose), polysorbate and related sugar solutions, emulsions, such as Intralipid® (Cutter

Laboratories, Inc., Berkley CA) and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Antioxidizing agents, such as but not limited to sodium bisulfite, sodium sulfite, or ascorbic acid, either alone or combined, are suitable stabilizing agents. Also useful are citric acid and its salts and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as but not limited to benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol.

The pharmaceutical compositions of the present invention also include compositions for delivery across cutaneous or mucosal epithelia including transdermal, intranasal, sublingual, buccal, and rectal administration. Such compositions can be part of a transdermal device, patch, topical formulation, gel, etc., with appropriate excipients. Thus, the compounds of the present invention are useful compounded with a penetration-enhancing agent such as 1-n-dodecylazacyclopentan-2-one or the other penetration-enhancing agents disclosed in U.S. Patent Nos. 3,991,203 and 4,122,170 which are hereby incorporated by reference in their entirety to describe penetration-enhancing agents which can be included in the transdermal or intranasal compositions of this invention.

Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, Mack Publishing Company, a standard reference text in this field, which is incorporated herein by reference in its entirety.

Useful pharmaceutical dosage-forms for administration of the compounds of this invention are illustrated as follows:

Capsules

A large number of unit capsules are prepared by filling standard two-piece hard gelatin capsules each with 0.1-50 milligrams of powdered active ingredient, 150 milligrams of lactose, 50 milligrams of cellulose, and 6 milligrams magnesium stearate.

Soft Gelatin Capsules

A mixture of active ingredient in a digestible oil such as soybean oil, lecithin, cottonseed oil or olive oil is prepared and injected by means of a positive displacement pump into gelatin to form soft gelatin capsules containing 0.1-50 milligrams of the active ingredient. The capsules are washed and dried.

Tablets

A large number of tablets are prepared by conventional procedures so that the dosage unit is 0.1-50 milligrams of active ingredient, 0.2 milligrams of colloidal silicon dioxide, 5 milligrams of magnesium stearate, 275 milligrams of microcrystalline cellulose, 11 milligrams of starch and 98.8 milligrams of lac ose. Appropriate coatings are applied to increase palatability or delay absorption.

Various modifications of the invention in addition to those shown and described herein are apparent to those skilled in the art from the foregoing description. Such modifications also are intended to fall within the scope of the appended claims.

The foregoing disclosure includes all the information deemed essential to enable those skilled in the art to practice the claimed invention. Because the cited patents or publications may provide further useful information these cited materials are hereby incorporated by reference in their entirety.

EXAMPLE 1
Synthesis of ROBA

A solution of retinoic acid (0.5 g, 1.67 mmol), iodomethylbutyrate (0.57 g, 1.5 eq) and triethylamine (0.35 mL, 1.5 eq) in acetonitrile was stirred at room temperature overnight. The product was detected by tlc (silica plate, EtOAc:hexane 1:4) as a deep yellow spot, Rf=0.7. The solvent was removed and the residue was dissolved in EtOAc and washed with 5% $NaHCO_3$ and then with water. The oily residue, obtained after drying and solvent removal, was separated on a silica gel column (EtOAc:hexane 1:4). The product was obtained as a yellow oil (0.43 g, 64%).

EXAMPLE 2
Synthesis of 13-*cis*-Retinoyloxy-(4-chlorophenyl)methyl butyrate

Triethylamine (1.2 eq) was added dropwise to a solution of 13-*cis*-retinoic acid (300 mg, 1 mmol) and 1-chloro-1-(4-chlorophenyl)methyl butyrate (1 eq) in dry DMF (1mL). The 1-chloro-1-(4-chlorophenyl)methyl butyrate was prepared from butyroyl chloride and p-chlorobenzaldehyde according to Rasmussen et al. (1967) J. Am. Chem. Soc. 89:5439. The solution was stirred at 70°C for several hours. When only a negligible amount of starting material could be detected by TLC (silica, ethyl acetate:hexane 1:4), the reaction was stopped. The mixture was dissolved in ether and washed with saturated NaCl. The ether solution was dried with $MgSO_4$ and the solvent removed by vacuum. The product was purified by chromatography on a silica gel column.

EXAMPLE 3

Cell Differentiation

The results of the effect on the level of differentiation (as presented by the % NBT reduction) in HL-60 cells, by butyric acid (BA) and retinoic acid (RA) was compared with ROBA in accordance with Tables 1 and 2 below.

Table 1: Differentiation of Promyelocytic Cells

concentration (μM)	BA (%)	RA (%)	ROBA (%)
0	7	7	7
0.1	4	5	12
0.2	5	6	20
0.5	4	5	40
1.0	6	21	81

The results of Table 1 show that compounds of the invention cause HL-60 cells to differentiate in a dose dependent manner, with an increase of 81% differentiated cells. This increase is much greater than any increase seen by BA alone or RA alone.

Table 2: Differentiation of Promyelocytic Cells

Concentration (μM)	NBT reduction (%)
0	7
RA (0.5)	5
BA (50)	15
BA (250)	11
BA (50) + RA (0.5)	29
BA (250) + RA (0.5)	11
ROBA (0.5)	40
ROBA (5)	84

The results in Table 2 show that compounds of the invention possess surprisingly superior activity than either BA alone or RA alone; and moreover, that the differentiating activity of ROBA is far greater than the combination of BA plus RA.

EXAMPLE 4

Additional Cell Differentiation Experiment

Human promyelocytic cell line HL-60 was grown for 4 days in the presence of 0.25 μM of RA, ROBA (RN-1), retinoyloxymethylpropionate (RN-2), retinoyloxymethylisobutyrate (RN-3) or retinoyloxymethylpivalate (RN-4). The compounds were synthesized as described in Example 1 or by appropriate modification of Example 1. Cell differentiation was measured by the NBT assay described above. The results of two separate experiments show that ROBA, RN-2 and RN-3 significantly increased the percentage of differentiated cells in the culture relative to RA or RN-4 (Fig. 1). Table 3 shows that the average percent differentiated cells for ROBA was 73% whereas the average percent differentiated cells for RN-4 was 17%. This difference is substantial and unexpected.

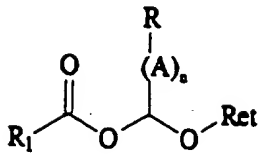
Table 3:ROBA and RN-4 Effective on Cell Differentiation

Compound	Exp A (%)[a]	Exp B (%)	Avg (%)
ROBA	13	21	17
RN-4	72	74	73

([a]) % differentiated cells as determined by NBT reduction.

WE CLAIM:

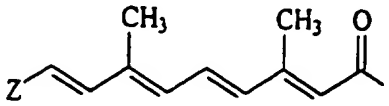
1. A compound of Formula (I):



Formula (I)

wherein:

Ret is selected from the group consisting of retinoyl group, a therapeutically-active retinoid carbonyl group, a carbonyl group represented by the formula



and retinoids which are C20 and C22 desmethyl vinylogs of said groups, wherein Z is a substituted or unsubstituted phenyl group, a substituted or unsubstituted naphthyl group or a cyclohexenyl group, and said phenyl or naphthyl group can be substituted with from 0 to 5 substitutents selected from the group consisting of halo, hydroxy, alkyl, alkyoxy, amino, cyano, carboxy or carbalkoxy, and wherein double bonds in the polyene chain of any of said groups can have a *cis* or *trans* configuration;

n is 0 or 1; and

when n is 0, then

R is H or C_1 to C_5 alkyl,

R_1 is ethyl, *n*-propyl or isopropyl,

with the proviso that when Ret is 13-*cis*-retinoyl and R_1 is *n*-propyl, then R cannot be H or C_2 to C_5 alkyl; or

when n is 1, then

R is aryl or heteroaryl optionally substituted with halo, hydroxy, alkyl, alkoxy, amino, cyano, carbalkoxy, nitro, or trifluoromethyl,

R_1 is C_2 to C_7 alkyl, optionally substituted by a phenyl or substituted phenyl group,

A is $(CH_2)_m$ or $(CH=CH)_m$,

m is 0 to 4; and

pharmaceutically acceptable salts thereof.

2. The compound of Claim 1 wherein the Ret group has a 13-*trans* double bond.

3. The compound of Claim 1 wherein the Ret group has a 9-cis double bond.

4. The compound of Claim 1 wherein R_1 is *n*-propyl.

5. The compound of Claim 4 which is 13-*trans*-retinoyloxymethyl butyrate.

6. The compound of Claim 4 which is 13-*trans*-retinoyloxy(p-chlorophenyl)methyl butyrate.

7. The compound of Claim 1 wherein R_1 is isopropyl.

8. The compound of Claim 7 which is 13-*trans*-retinoyloxymethyl isobutyrate.

9. The compound of Claim 7 which is 1-(13-*trans*-retinoyloxy-2-(3-pyridyl)ethyl isobutyrate.

10. A pharmaceutical composition useful for the treatment of disorders including cancer and other proliferative diseases comprising a therapeutically effective amount of a compound of any one of Claims 1 to 9 and a pharmaceutically effective carrier or diluent.

11. A pharmaceutical composition useful as a differentiating agent or an anti-proliferation agent comprising a therapeutically-effective amount of a

compound of any one of Claims 1 to 9 and a pharmaceutically-effective carrier or diluent.

12. A method for treating, preventing or ameliorating cancer or other proliferative disorder in a subject in need of such treatment which comprises administering an amount of a compound of any one of Claims 1 to 9 effective to treat, prevent or ameliorate said cancer or said disorder in a subject.

13. The method of Claim 12 wherein said disorder is leukemia, squamous cell carcinoma, neuroblastoma renal cell carcinoma, melanoma or pancreatic carcinoma.

14. The method of Claim 12 or 13 wherein said effective amount is an amount effective to inhibit histone deacetylase.

15. A method of differentiating or blocking proliferation of cancerous or neoplastic cells comprising administering to said cells an amount of a compound of any one of Claims 1 to 9 effective to cause differention of or to block proliferation of cancerous or neoplastic cells.

16. The method of Claim 15 wherein said cells are *in vivo*.

17. The method of Claim 15 wherein said cells are *in vitro*.

18. A method for enhancing the actions of a pharmaceutical agent useful for the treatment of cancer and other proliferative disorders, comprising co-administering a therapeutically-effective amount of a compound of any one of Claims 1 to 9, with a therapeutically-effective amount of said pharmaceutical agent.

19. The method of Claim 18 wherein said pharmaceutical agent comprises a differentiating agent, a cytokine, an interleukin, an anti-cancer agent, a chemotherapeutic agent, an antibody, a conjugated

antibody, a hormone antagonist, an immune stimulant, an antibiotic, or a growth stimulant.

20. The method of Claim 19, wherein said antibiotic selected from the group consisting of ganciclovir, acyclovir, and famciclovir.

21. The method of Claim 19, wherein said chemotherapeutic agent is selected from the group consisting of an alkylating agent, a purine analog, a pyrimidine analog, a vinca alkaloid, a vinca-like alkaloid, etoposide, an etoposide-like drug, a corticosteroid, a nitrosourea, an antimetabolite, a platinum-based cytotoxic drug, a hormonal antagonist, an anti-androgen and an anti-estrogen.

22. The method of Claim 19 wherein said cytokine is an interferon.

23. The method of Claim 19 wherein said immune stimulant is *Corynebacterium parvum* or a sarcolectin.

24. The method of Claim 19 wherein the chemotherapeutic agent is selected from the group consisting of tamoxifen, doxorubicin, L-asparaginase, dacarbazine, amascrine, procarbazine, hexamethylmelamine, mitosantrone and gemcitabine.

25. A method of treating gastrointestinal disorders which comprises administering a therapeutically-effective amount of a compound of any one of Claims 1 to 9 to a subject.

26. A method of treating cutaneous ulcers which comprises administering a therapeutically-effective amount of a compound of any one of Claims 1 to 9 to a subject.

27. A method of inducing wound healing which comprises administering a therapeutically-effective amount of a compound of any one of Claims 1 to 9 to a subject.

28. A method of treating, preventing or ameliorating cancer or other proliferative disorder in a subject in need of such treatment which comprises administering an amount of a compound of any one of Claims 1 to 9 effective to induce cellular apoptosis of the cancer cells or of the cells of the proliferative disorder.

29. A method of inhibiting histone deacetylase in cells which comprises administering an effective amount of a compound of any one of Claims 1 to 9 to said cells.

30. The method of Claim 29 wherein said cells are *in vivo*.

31. The method of any one of Claims 12 to 30 wherein said compound is administered orally, parenterally, transdermally or transmucosally.

32. A method of ameliorating wrinkles which comprises administering a therapeutically-effective amount of a compound of any one of Claims 1 to 9 to a subject.

33. A method of treating or ameliorating a dermatological disorder which comprises administering an effective amount of a compound of any one of Claims 1 to 4 or 7 wherein n is 1 to a patient.

34. The method of Claim 33 wherein said compound is 13-*trans*-retinoyloxy(p-chlorophenyl)methyl butyrate or 1-(13-*trans*- retinoyloxy-2-(3-pyridyl)ethyl isobutyrate.

35. The method of Claim 33 or 34 wherein said dermatological disorder is psoriasis or acne.

36. The method of any one of Claims 32-35 wherein said compound is administered topically.

37. A pharmaceutical composition comprising a therapeutically-effective amount of a compound of any one of Claims 1 to 9 with a therapeutically-effective amount of a pharmaceutical agent, wherein said agent is selected from the group consisting of a cytokine, an interleukin,

an anti-cancer agent or anti-neoplastic agent, a chemotherapeutic agent, an antibody, a conjugated antibody, an immune stimulant, an antibiotic, a hormone antagonist or a growth stimulant.

38. The composition of Claim 37 wherein said pharmaceutical agent comprises a cytotoxic agent.

39. The composition of Claim 37 wherein said antibiotic is an antiviral nucleoside antibiotic selected from the group consisting of ganciclovir, acyclovir, and famciclovir.

40. The composition of Claim 37 wherein said antibiotic is ganciclovir.

41. The composition of Claim 37 wherein said chemotherapeutic agent is selected from the group consisting of alkylating agents, purine and pyrimidine analogs, vinca and vinca-like alkaloids, etopsides and etopside-like drugs, corticosteroids, nitrosoureas, antimetabolites, platinum based cytotoxic drugs, hormonal antagonists, anti-androgens and antiestrogens.

42. The composition of Claim 37 wherein said cytokine is an interferon.

43. The composition of Claim 37 wherein said immune stimulant is *Corynebacterium parvum* or a sarcolectin.

44. The composition of Claim 37, wherein the chemotherapeutic agent is selected from the group consisting of tamoxifen, doxorubicin, L-asparaginase, dacarbazine, amsacrine, procarbazine, hexamethylmelamine, mitoxantrone and gemcitabine.

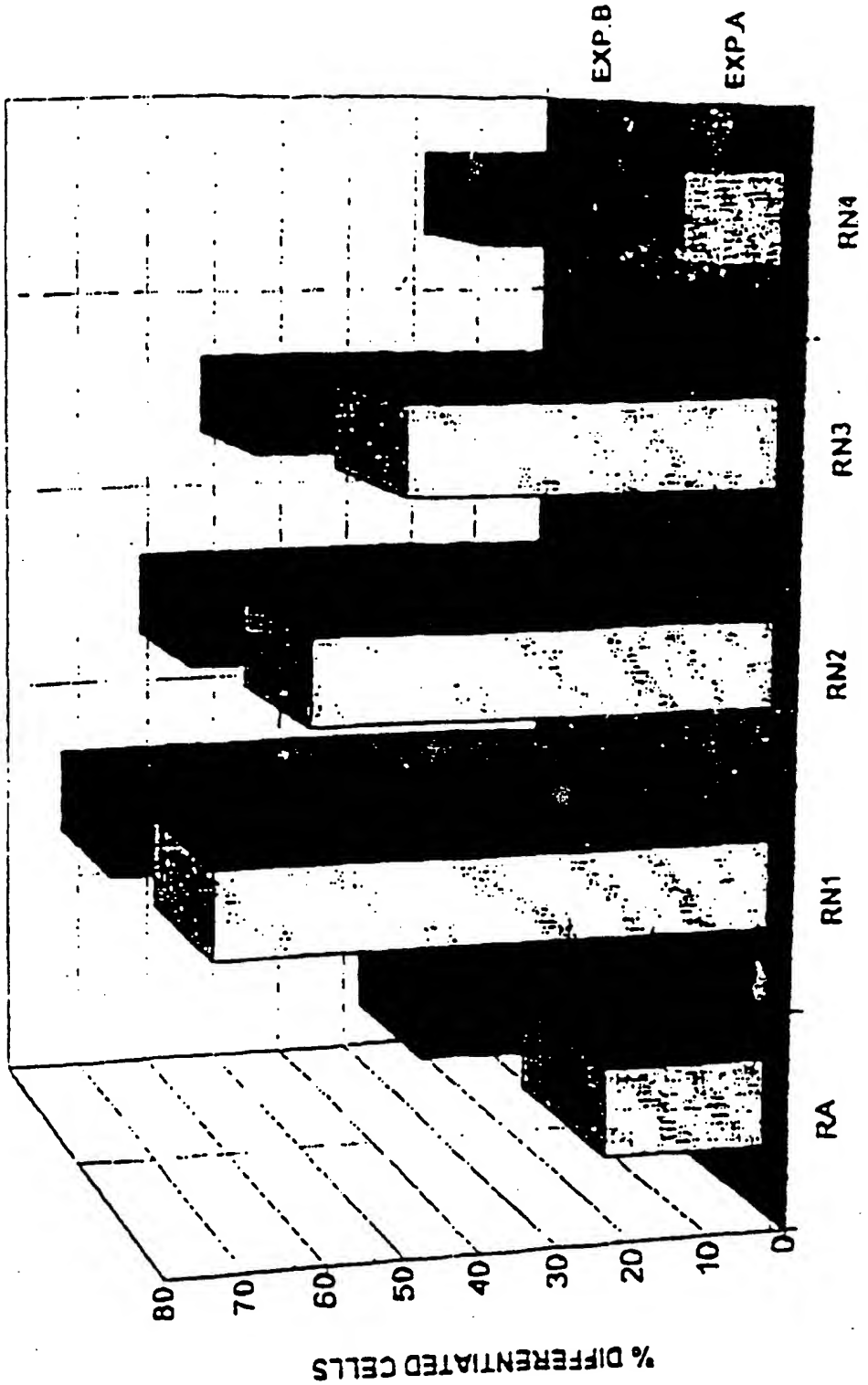


Fig. 1: THE EFFECT OF ALL-TRANS RETINOIDS ON DIFFERENTIATION ON HUMAN LEUKEMIC CELLS

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/11452

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 31/225; C11C 3/00; C07C 403/10
US CL :514/548; 554/221
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/548; 554/221

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,900,478 (GROSS) 13 February 1990 (13/02/90), see entire document, especially column 1.	1-8, 10-12, 27, 32-34

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
22 AUGUST 1997

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Form PCT/ISA/210 (second sheet)(July 1992)*

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International application No.
PCT/US97/11452

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-13, 15-17, 28 and 37-44, drawn to a compound, composition and a method of use for treating, preventing or ameliorating cancer or other proliferative disorder.

Group II, claims 18-24, drawn to a method of use for enhancing the actions of a pharmaceutical agent.

Group III, claim 25, drawn to a method of use for treating gastrointestinal disorders.

Group IV, claim 26, drawn to a method of use for treating cutaneous ulcers.

Group V, claim 27, drawn to a method of use for inducing wound healing.

Group VI, claims 29 and 30, drawn to a method of use for inhibiting histone deacetylase.

Group VII, claim 32, drawn to a method of use for ameliorating wrinkles.

Group VIII, claims 33 and 34, drawn to a method of use for treating or ameliorating a dermatological disorder.

The inventions listed as Groups I-VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Applicants are claiming various methods of use for the compounds found in claim 1 that are so diverse that they do not represent a single inventive concept. For example, the method of use in Group I is for treating, preventing or ameliorating cancer or other proliferative disorder by admininstering a compound found in any one of claims 1-9. The method of use claimed in Group III is for treating gastrointestinal disorders by administering a compound of any one of claims 1-9. Since the methods of using the compounds in claims 1-9 are so diverse, unity is lacking.